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(54) **METHOD FOR OBTAINING POLYPEPTIDES IN A CELL-FREE SYSTEM**

VERFAHREN ZUR HERSTELLUNG VON POLYPEPTIDEN IN EINEM ZELLFREIEN SYSTEM

PROCEDE D'OBTENTION DE POLYPEPTIDES DANS UN SYSTEME EXEMPT DE CELLULES

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Description

Field of the Art

This invention relates to molecular biology and bio-engineering, and more particularly to methods of preparing polypeptides in a cell-free translation system.

Polypeptides are widely used in medicine as regulators of biological processes. Known in the prior art are, e.g., polypeptides activating the immune system, polypeptides which are neuromediators and transmitters, polypeptides regulating salt metabolism, etc. Polypeptides are also used in agriculture as biological stimulants, e.g., growth hormones. They are also used in bioelectronics, e.g., as rodopsin films.

State of the Art

Known in the art is a method for a preparative expression of cell genetic material by a method of genetic engineering based on the introduction of a foreign DNA into a live cell, the genetic material of said foreign DNA being expressed by the apparatus of the host cell. This method is widely employed in commercial production of proteins.

However, the above method has a limited application. This is associated with the complexity of isolation of the products of gene expression by the transformed cells, lethality of some specific products to the host cell, elimination of the transformed plasmids from the cell, and proteolytic degradation or aggregation of the products of expression of the foreign gene.

It follows from the foregoing that the method of genetic engineering does not provide for possibilities of a preparative expression of all genes.

Known is another method of expression of genes based on the use of a cell-free system of continuous conjugated transcription/translation (Gene, 1989, v. 84, p. 463). This system is free of limitations imposed by a cell and ensures expression of substantially any gene in the form of a DNA molecule engineered in the required manner.

However, this method cannot be applied for cell-free eukaryotic systems. The problem is that upon expression of genes by the method, use is made of endogenous RNA-polymerases of the cells employed for preparation of the cell-free system of conjugated transcription/translation. This requires the use of special methods for isolation of the cell extract to ensure the maintenance of the activity of endogenous RNA-polymerases. Moreover, in eukaryotic cells the transcription and translation processes are, as a rule, dispersed in space and time: the transcription takes place in the cell nucleus, while the translation occurs in the cell cytoplasm after relevant modifications of mRNA. Therefore up to the present all attempts to obtain a reliable system of conjugated transcription/translation based on eukaryotic cell extracts have been unsuccessful. The only method

providing a reliable preparation of such extracts is based on the preparation of the S30 extract from bacterial cells of *Escherichia coli*. Besides the plasmid containing a gene coding for a specific product has a selection gene (a gene providing resistance to the action of an antibiotic) which is also controlled by a promoter of the RNA-polymerase of *E. coli* and is expressed as efficiently as the gene coding for the specific product. As a result, in addition to the specific product, a side product is synthesized upon functioning of the system.

Known in the art is one more method of preparative synthesis of polypeptides based on the use of a continuous cell-free translation system containing RNA as the nucleic acid template. The method consists of preparing polypeptides on ribosomes in a cell-free translation system containing ATP, GTP and amino acids as substrates accompanied by the formation of translation products in the system which include the specific product, AMP, GDP, pyrophosphate and inorganic phosphate. In the process of translation, translation products, including AMP, GDP, pyrophosphate, inorganic phosphate and the specific product, are removed from the system as substrates are converted to products with a simultaneous delivery in the system of substrates in the form of amino acids, ATP, GTP to maintain their initial concentration unchanged (Science, 1988, v. 242, p. 1162).

This method makes it possible to carry out the preparative synthesis of substantially any polypeptide in a cell-free translation system prepared from cells of any organism.

However, the application of this method makes impossible the expression of the genetic material as DNA molecules, since a template RNA is used in this method. This means that to realize the method, it is necessary to carry out an additional synthesis of template RNAs. As known, a template RNA is obtained from DNA molecules using transcription by RNA-polymerases. This is a labor-consuming and expensive process. Thus, at present the available methods do not permit the synthesis of polypeptides using DNA molecules as templates in a cell-free system.

Disclosure of the Invention

The object of the invention is to develop such a method of preparation of polypeptides in cell-free systems which would ensure preparation of polypeptides with the use of DNA molecules in any cell-free system based on both prokaryotic and eukaryotic extracts.

The present invention thus provides a cell-free method for synthesizing a polypeptide from an RNA transcript, by adding to a vessel containing an ultrafiltration membrane barrier, a mixture comprising a eukaryotic cell extract capable of supporting *in vitro* translation, ATP, GTP, CTP, UTP, and amino acids comprising:

continuously delivering to the vessel the substrates

ATP, GTP, CTP, UTP and amino acids at a rate that maintains their initial concentration in the vessel and continuously removing from the vessel, through the ultrafiltration barrier, the products of the process, including AMP, GDP, CDP, UDP, pyrophosphate, inorganic phosphate, and said polypeptide; wherein an exogenous bacteriophage RNA polymerase and a DNA molecule which encodes said polypeptide and contains a promoter site specific to said exogenous RNA polymerase is added to the mixture in order to generate said RNA transcript.

Prokaryotic and eukaryotic cell-free translation systems are used as cell-free translation systems according to the invention. For example systems based on *E. coli* extracts can be used as prokaryotic cell-free systems, and systems based on extracts from wheat embryos or on lysates from *E. coli* rabbit reticulocytes can be used as eukaryotic cell-free systems. The ratio of the components in the reaction mixture, ion and temperature conditions of the synthesis are optimal for the organisms from which cell-free systems and exogenous RNA-polymerases are prepared. The range of these conditions is rather wide.

The method implies the use of an exogenous phage RNA-polymerase, e.g., phage T7 RNA-polymerase or phage SP6 RNA-polymerase, as an exogenous RNA-polymerase.

As said, in some cases it is expedient to use a prokaryotic cell-free system based on *E. coli* extracts. Such a translation system contains an endogenous RNA-polymerase. To prevent the formation of additional translation products, an additional delivery of an inhibitor of the endogenous RNA-polymerase should be used. E.g., rifampicin is used as an inhibitor of the prokaryotic endogenous RNA-polymerase.

The nucleic acid employed in the system is represented by the protein-coding genes in the form of DNA molecules with promoter sites specific to an exogenous RNA-polymerase. Such protein-coding genes can be contained in e.g., DNA molecules obtained by amplification of a DNA fragment or a plasmid DNA.

The proposed method has no disadvantages of the previously discussed genetic engineering method and known methods of preparative synthesis of polypeptides in continuous cell-free translation systems. It provides for the preparation of polypeptides within various cell-free systems without a preliminary synthesis of template RNA molecules. Due to the choice of the components, the synthesis of template RNA proceeds directly in the cell-free system.

The proposed method ensures the preparative synthesis of polypeptides at a constant rate during tens of hours with a yield of the functionally active product (polypeptide) of 1 to 10 nmol per 1 ml of the reaction mixture and can be employed in commercial production of polypeptides in cell-free systems.

Description of the Drawings

The invention will further be described with reference to the appended drawings in which:

Figs. 1, 2, 4, 5 and 7 represent graphic dependencies of the quantity of synthesized polypeptide in nanomoles versus the time of synthesis, in hours.

Fig. 3 is a photograph of the fluorogram of an SDS-urea-polyacrylamide gel illustrating the distribution of the translation products according to their molecular weights.

Fig. 6 is a photograph of a thin-layer chromatography autoradiograph illustrating the distribution of the products of the reaction catalyzed by chloramphenicol acetyl transferase enzyme (Anal. Biochem., 1987, v. 160, p. 65-67).

Preferable Variant of Embodiment of the Invention

The technological aspect of the method of preparing polypeptides in cell-free translation systems is simple and the method can be realized as follows.

Extracts of prokaryotic and eukaryotic cells containing ribosomes and all components of the translation machinery but free of endogenous mRNA and DNA are prepared using known methods. Low molecular weight components including amino acids, ATP, GTP, CTP, UTP, exogenous RNA-polymerase, the gene in the form of a DNA molecule with a promoter site specific to the above polymerase are added to the extract.

The cell-free system is protected from the environment by a porous barrier with pores sufficient to allow passage of specific products. The reaction vessel for the synthesis of polypeptides can be a reservoir in which the cell-free system protected by a porous barrier is placed. A porous barrier can be made either of organic or inorganic material. E.g., ultrafiltration membranes, hollow fibers, microcapsules or films whose shell represents polyelectrolyte complexes can be used as porous barriers. The cell content is then heated to the required temperature.

During the synthesis, the translation products are removed from the reaction vessel through the porous barrier. Simultaneously, substrates from a separate reservoir are supplied into the system to maintain their initial concentrations. The specific product withdrawn from the system is concentrated and purified.

The method is illustrated by the following examples:

Example 1.

1 ml of the reaction mixture contained the following: 350 μ l of the S30 extract from *E. coli*, 0.1 mg of tRNA, 0.04 mg of a DNA fragment containing the gene for the precursor of β -lactomase and the promoter for T7 polymerase obtained according to the technique described (Molecular Cloning, 1989, Cold Spring Harbor Laboratory Press, ed. J. Sambrook, E.F. Fritsch, T. Ma-

niatis, p. 1-21), 30,000 U of T7 polymerase, 0.1 mg of pyruvate kinase, 50 U of ribonuclease inhibitor from human placenta, 5 µg of each of the protease inhibitors (leupeptin, chymostatin) and α2-macroglobulin in buffer A: 50 mM Tris Ac, pH 7.5, 14 mM MgCl₂, 100 mM KAc, 2 mM CaAc₂, 1 mM ATP, 0.4 mM GTP, 0.4 mM CTP, 0.4 mM UTP, 10 mM phosphoenolpyruvate, 4 mM dithiothreitol, 50 µM spermidine, 10 µg leukovorin, 40 µM rifampicin, 30 µM [³H]Leu with specific activity 1.7 Ci/mmol and 30 µM each of the other 19 amino acids.

0.5 ml of the cell-free system was placed in a cell for ultra-filtration and the peptide was synthesized at 37°C. The translation products, including the specific product and the products of decomposition, were withdrawn through a porous barrier with a simultaneous delivery of substrates in the form of ATP, GTP, CTP, UTP and amino acids in buffer A over a period of 20 hours. As a result, protein (β-lactomase) was obtained. The substrates were supplied at a rate of 2 ml/h.

During the entire synthesis, the specific product was synthesized at a constant rate. The dependence of the amount of product on the time of synthesis is shown in Fig. 1. The abscissa axis shows the time in hours and the ordinate axis shows the amount of the obtained product in nanomoles. As a result, 250 pmol of β-lactomase was synthesized during the 20 hours of system operation.

Example 2.

1 ml of the reaction mixture contained the following: 350 µl of the S30 extract from *E. coli*, 0.2 mg of tRNA, 0.1 mg of plasmid containing the gene for dihydrofolate reductase containing a promoter site recognized by polymerase and obtained according to the technique described (Molecular Cloning, 1989, Cold Spring Harbor Laboratory Press, ed. J. Sambrook, E.F. Fritsch, T. Maniatis, p. 1-21), 20,000 U of SP6 polymerase, 0.1 mg of pyruvate kinase, 50 U of ribonuclease inhibitor from human placenta, 5 µl of each protease inhibitors (leupeptin, chymotrypsin) and α2-macroglobulin in buffer A: 50 mM Tris-Ac, pH 7.5, 14 mM MgCl₂, 100 mM KAc, 2 mM CaAc₂, 1 mM ATP, 0.4 mM GTP, 0.4 mM CTP, 0.4 mM UTP, 10 mM phosphoenolpyruvate, 4.0 mM dithiothreitol, 50 µM spermidine, 10 µg leukovorin, 40 µM rifampicin, 30 µM [³⁵S]Met with specific radioactivity of 900 mCi/mmol and 30 µM of each of the other 19 amino acids.

0.5 ml of the cell-free system was placed in an ultrafiltration cell and the polypeptide was synthesized at 37°C. The translation products, including the specific product and the products of decomposition, were removed from the system through a porous barrier with simultaneous delivery of substrates such as ATP, GTP, CTP, UTP and amino acids in buffer A, into the reaction mixture, over the course of 20 hours. As a result, dihydrofolate reductase was obtained. The substrates were delivered at a rate of 1.5 ml/h.

During the course of the synthesis, the specific product was synthesized at a constant rate. The dependence of the amount of the product obtained on the time of the synthesis is given in Fig. 2. The abscissa axis shows the time of the synthesis in hours and the ordinate axis shows the amount of the synthesized product in nanomoles.

As a result, 680 pmol of dihydrofolate reductase was synthesized during 24 hours. The synthesized enzyme was active functionally. Its specific activity was measured as described (Nature, 1960, v. 188, p. 231-232) and was $0.13 \cdot 10^{-4}$ activity units per picomole of the enzyme synthesized.

In this case, the plasmid utilized contained the gene for dihydrofolate reductase under the control of a promoter site recognized by SP6 polymerase and the gene for β-lactomase under the control of a promoter site recognized by *E. coli* RNA-polymerase. Since rifampicin, an inhibitor of *E. coli* RNA-polymerase, was present in the system, no synthesis of β-lactomase occurred. Therefore, only the specific product dihydrofolate reductase was synthesized in the system.

The results of the electrophoretic analysis of the polypeptide obtained at 2, 4, 6, 8 and 10 hours after the beginning of system operation are represented in Fig. 3.

Example 3.

1 ml of the incubation mixture contained the following: 320 µl of wheat embryo extracts, 0.1 mg of the plasmid with the gene for dihydrofolate reductase carrying a promoter site specific to SP6 polymerase obtained by the method described (Molecular Cloning, 1989, Cold Spring Harbor Laboratory Press, ed. J. Sambrook, E.F. Fritsch, T. Maniatis, p. 1-21), 20,000 U of SP6 polymerase, 0.1 mg of pyruvate kinase, 50 U of ribonuclease inhibitor from human placenta, 5 µg of each of the protease inhibitors (leupeptin, chymotrypsin) and α2-macroglobulin in buffer A: 40 mM HEPES, pH 7.6, 2.5 mM MgAc₂, 70 mM KAc, 1 mM ATP, 0.4 mM GTP, 0.4 mM CTP, 0.4 mM UTP, 0.25 mM spermidine, 4.0 mM dithiothreitol, 6 mM creatin phosphate, 20 µM [¹⁴C]Leu with specific radioactivity of 21 µCi/mmol, 20 µM of each of the other 19 amino acids.

0.5 ml of the cell-free system was placed in a cell for ultrafiltration and the polypeptide was synthesized at 24°C. The translation products, including the specific product and the products of decomposition, were removed from the system through a porous barrier with a simultaneous delivery of substrates such as ATP, GTP, CTP, UTP and amino acids in buffer A into the reaction mixture during 24 hours. As a result, dihydrofolate reductase was obtained. The substrates were delivered at a rate of 2.0 ml/h.

During the entire synthesis the product was synthesized at a constant rate. The dependence of the amount of the product obtained on the time of the synthesis is given in Fig. 4. The abscissa axis shows the time of the

synthesis in hours and the ordinate axis shows the amount of the product obtained in nanomoles. As a result, 5 nmol of dihydrofolate reductase were synthesized. The synthesized enzyme was active functionally. The specific activity of the enzyme obtained was measured as described (Nature, 1960, v. 188, p. 231-232). It was $0.25 \cdot 10^{-4}$ activity units per picomole of the enzyme synthesized.

Example 4

1 ml of the incubation mixture contained: 600 μ l of lysate from rabbit reticulocytes, 0.1 ml of plasmid containing the gene for chloramphenicol acetyl transferase under the control of a promoter specific to SP6 polymerase and obtained according to the method described (Molecular Cloning, 1989, Cold Spring Harbor Laboratory Press, ed. J. Sambrook, E.F. Fritsh, T. Maniatis, p. 1-21), 30,000 U of SP6 polymerase, 0.1 mg of pyruvate kinase, 50 U of the ribonuclease inhibitor from human placenta, 5 μ g of each of the protease inhibitors (leupeptin, chymotrypsin) and α 2-macroglobulin in buffer A: 25 mM HEPES, pH 7.6, 1.5 mM $MgAc_2$, 100 mM KAc, 1 mM ATP, 0.4 mM GTP, 0.4 mM CTP, 0.4 mM UTP, 0.25 mM spermidine, 4.0 mM dithiothreitol, 6 mM creatin phosphate, 20 μ M [^{35}S]Met with specific radioactivity of 800 mCi/mmol, 20 μ M of each of the other 19 amino acids.

0.5 ml of the cell-free system was placed in a cell for ultrafiltration and the polypeptide was synthesized at 37°C. The translation products, including the specified product and the products of decomposition, were removed through a porous barrier with a simultaneous delivery of substrates such as ATP, GTP, CTP, UTP and amino acids in buffer A into the reaction mixture during 34 hours. As a result, chloramphenicol acetyl transferase was obtained. The substrates were delivered at a rate of 1.5 ml/h.

During the entire synthesis the specific product was synthesized at a constant rate. The dependence of the amount of the product obtained on the time of the synthesis is given in Fig. 5. The abscissa axis shows the time of synthesis in hours and the ordinate axis shows the amount of the product obtained in nanomoles. As a result, 2.5 nmol of chloramphenicol acetyl transferase was synthesized. The enzyme synthesized was active functionally. The functional activity of the enzyme obtained was measured as described (Anal. Biochem., 1987, v. 160, p. 65-67). The results of the analysis of functional activity of the enzyme obtained in 0, 0.5, 5, 7, 9 and 12 hours performed using thin-layer chromatography followed by autoradiography are represented in Fig. 6.

Example 5

1 ml of the incubation mixture contained: 600 μ l of lysate from rabbit reticulocytes, 0.1 mg of plasmid con-

taining the gene for dihydrofolate reductase containing a promoter recognized by SP6 polymerase and obtained according to the method described (Molecular Cloning, 1989, Cold Spring Harbor Laboratory Press, ed. J. Sambrook, E.F. Fritsh, T. Maniatis, p. 1-21), 30,000 U of SP6 polymerase, 0.1 mg of pyruvate kinase, 50 U of the ribonuclease inhibitor from human placenta, 5 μ g of each of the protease inhibitors (leupeptin, chymotrypsin) and α 2-macroglobulin in buffer A: 25 mM HEPES, pH 7.6, 1.5 mM $MgAc_2$, 100 mM KAc, 1 mM ATP, 0.4 mM GTP, 0.4 mM CTP, 0.4 mM UTP, 0.25 mM spermidine, 4.0 mM dithiothreitol, 6 mM creatin phosphate, 20 μ M [^{14}C]Leu with specific radioactivity of 21 mCi/mmol, 20 μ M each of the other 19 amino acids.

0.5 ml of the cell-free system was placed in a cell for ultrafiltration and the polypeptide was synthesized at 37°C. The translation products, including the specific product and the products of decomposition were removed through a porous barrier with a simultaneous delivery of substrates such as ATP, GTP, CTP, UTP and amino acids in buffer A during 20 hours. As a result, dihydrofolate reductase was obtained. The substrates were delivered at a rate of 2.0 ml/h.

During the entire synthesis the product was synthesized at a constant rate. The dependence of the amount of the product obtained on the time of the synthesis is given in Fig. 7. The abscissa axis shows the time of the synthesis in hours and the ordinate axis shows the amount of the product obtained in nanomoles. As a result, 7.0 nmol of dihydrofolate reductase was synthesized. The enzyme synthesized was active functionally. The specific activity of the enzyme obtained was measured as described (Nature, 1960, v. 188, p. 231-232) and was determined to be $0.3 \cdot 10^{-4}$ activity units per picomole of the enzyme synthesized.

Industrial Applicability

The polypeptide obtained according to the invention can be used in medicine, agriculture, and bioelectronics. The method is a multi-purpose one as it ensures synthesis of a template RNA in the process of polypeptide preparation.

Claims

1. A cell-free method for synthesizing a polypeptide from an RNA transcript, by adding to a vessel containing an ultrafiltration membrane barrier, a mixture comprising a prokaryotic or a eukaryotic cell extract capable of supporting in vitro translation, ATP, GTP, CTP, UTP, and amino acids comprising;

continuously delivering to the vessel the substrates ATP, GTP, CTP, UTP and amino acids at a rate that maintains their initial concentration in the vessel and continuously removing

from the vessel, through the ultrafiltration barrier, the products of the process, including AMP, GDP, CDP, UDP, pyrophosphate, inorganic phosphate and said polypeptide; wherein an exogenous bacteriophage RNA polymerase and a DNA molecule which encodes said polypeptide and contains a promoter site specific to said exogenous RNA polymerase is added to the mixture in order to generate said RNA transcript.

2. The method of claim 1, wherein said extract is substantially free of endogenous mRNA and DNA.
3. The method of claim 2, wherein the phage RNA-polymerase is SP6 RNA-polymerase.
4. The method of claim 2, wherein the phage RNA-polymerase is T7 RNA-polymerase.
5. The method of any one of claims 1 to 4 wherein the DNA molecule is a plasmid DNA molecule.
6. The method of any one of claims 1 to 5 wherein the DNA molecule is produced by amplification of a gene encoding the desired polypeptide.
7. The method of any one of claims 1 to 6 wherein the eukaryotic cell extract is a plant cell extract.
8. The method of claim 7, wherein the plant cell extract is a wheat embryo extract.
9. The method of any one of claims 1 to 6 wherein the eukaryotic cell extract is an animal cell extract.
10. The method of claim 9, wherein the animal cell extract is a rabbit reticulocyte lysate.

Patentansprüche

1. Zell-freies Verfahren zur Synthese eines Polypeptids von einem RNA-Transkript, indem einem eine Ultrafiltrationsmembranbarriere enthaltenden Gefäß eine einen prokaryotischen oder eukaryotischen Zellextrakt, der zur Unterstützung einer *in vitro*-Translation in der Lage ist, ATP, GTP, CTP, UTP und Aminosäuren beinhaltende Mischung hinzugegeben wird, umfassend:

Kontinuierliches Zuführen der Substrate ATP, GTP, CTP, UTP und von Aminosäuren in das Gefäß mit einer Rate, die zum Erhalt ihrer anfänglichen Konzentration im Gefäß führt und kontinuierliches Entfernen der Produkte des Verfahrens, einschließlich AMP, GDP, CDP, UDP, Pyrophosphat, anorganisches Phosphat

und des Polypeptids, aus dem Gefäß durch die Ultrafiltrationsbarriere;

wobei eine exogene Bakteriophagen-RNA-Polymerase und ein DNA-Molekül, welches das Polypeptid codiert und eine für die exogene RNA-Polymerase spezifische Promotorstelle aufweist, der Mischung hinzugesetzt werden, um das RNA-Transkript zu erzeugen.

2. Verfahren von Anspruch 1, wobei der Extrakt im wesentlichen frei von endogener mRNA und DNA ist.
3. Verfahren von Anspruch 2, wobei die Phagen-RNA-Polymerase SP6 RNA-Polymerase ist.
4. Verfahren von Anspruch 2, wobei die Phagen-RNA-Polymerase T7 RNA-Polymerase ist.
5. Verfahren nach einem der Ansprüche 1 bis 4, wobei das DNA-Molekül ein Plasmid-DNA-Molekül ist.
6. Verfahren nach einem der Ansprüche 1 bis 5, wobei das DNA-Molekül durch Amplifizierung eines das gewünschte Polypeptid codierenden Gens hergestellt wird.
7. Verfahren nach einem der Ansprüche 1 bis 6, wobei der eukaryotische Zellextrakt ein Pflanzenzellextrakt ist.
8. Verfahren nach Anspruch 7, wobei der Pflanzenzellextrakt ein Weizenkeimextrakt ist.
9. Verfahren nach einem der Ansprüche 1 bis 6, wobei der eukaryotische Zellextrakt ein Tierzellextrakt ist.
10. Verfahren nach Anspruch 9, wobei der Tierzellextrakt ein Kaninchenreticulocytlisat ist.

Revendications

1. Méthode acellulaire de synthèse d'un polypeptide à partir d'un transcrit d'ARN, en ajoutant dans un récipient contenant une barrière constituée par une membrane pour ultrafiltration, un mélange comprenant un extrait de cellules procaryotes ou eucaryotes capable d'effectuer la traduction *in vitro*, ATP, GTP, CTP, UTP, et des acides aminés, comprenant l'étape consistant à :

fournir continuellement dans le récipient les substrats ATP, GTP, CTP, UTP et des acides aminés à une vitesse permettant de conserver leur concentration initiale dans le récipient et à retirer continuellement du récipient, à travers la barrière pour ultrafiltration, les produits du pro-

cédé, comprenant AMP, GDP, CDP, UDP, du pyrophosphate, du phosphate inorganique et ledit polypeptide ;

où une ARN polymérase exogène de bactériophage et une molécule d'ADN qui code pour ledit polypeptide et qui contient un site promoteur spécifique pour ladite ARN polymérase exogène sont ajoutées au mélange afin de générer ledit transcrit d'ARN.

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2. Méthode selon la revendication 1, dans laquelle ledit extrait est sensiblement dépourvu d'ARNm et d'ADN endogènes.

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3. Méthode selon la revendication 2, dans laquelle l'ARN polymérase phagique est l'ARN polymérase de SP6.

4. Méthode selon la revendication 2, dans laquelle l'ARN polymérase phagique est l'ARN polymérase de T7.

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5. Méthode selon l'une quelconque des revendications 1 à 4, dans laquelle la molécule d'ADN est une molécule d'ADN plasmidique.

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6. Méthode selon l'une quelconque des revendications 1 à 5, dans laquelle la molécule d'ADN est produite par amplification d'un gène codant pour le polypeptide souhaité.

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7. Méthode selon l'une quelconque des revendications 1 à 6, dans laquelle l'extrait de cellules eucaryotes est un extrait de cellules végétales.

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8. Méthode selon la revendication 7, dans laquelle l'extrait de cellules végétales est un extrait d'embryons de blé.

9. Méthode selon l'une quelconque des revendications 1 à 6, dans laquelle l'extrait de cellules eucaryotes est un extrait de cellules animales.

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10. Méthode selon la revendication 9, dans laquelle l'extrait de cellules animales est un lysat de réticulocytes de lapin.

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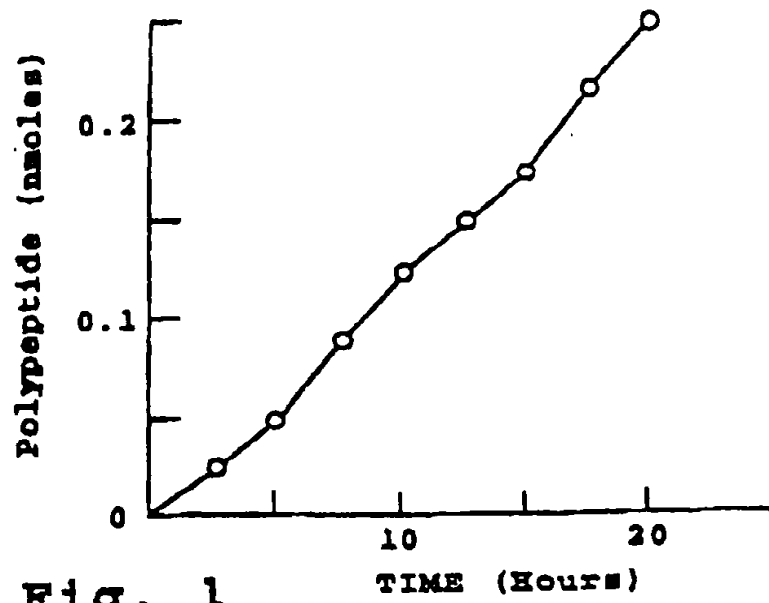


Fig. 1

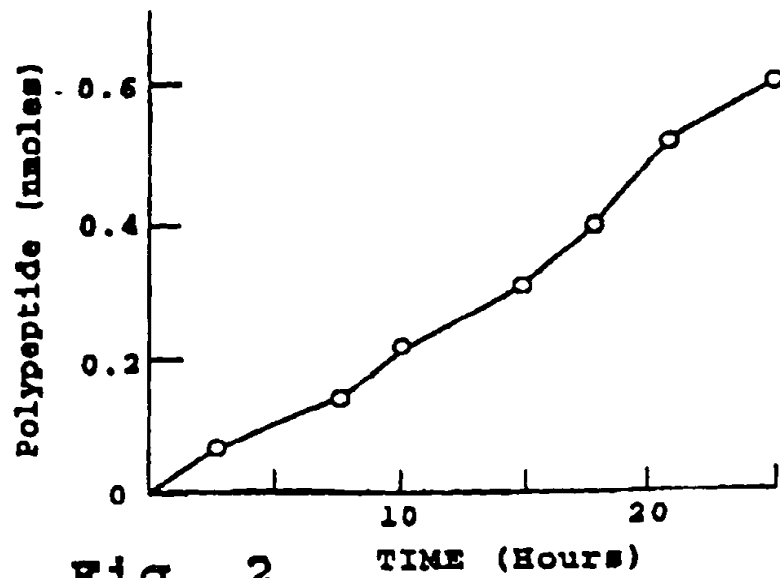


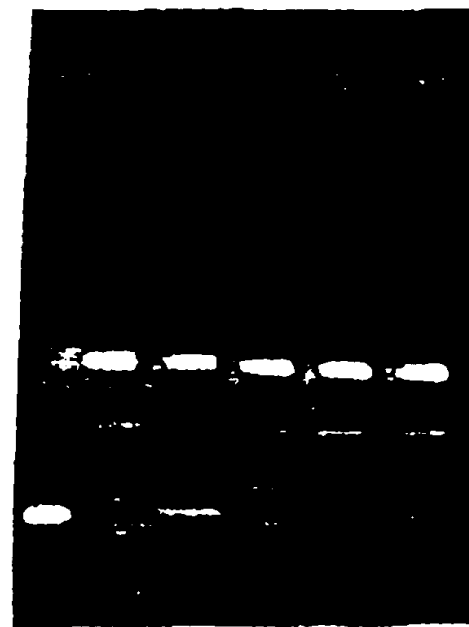
Fig. 2



Fig. 3

2 4 6 8 10

Fig. 6



0 0.5 5 7 9 12

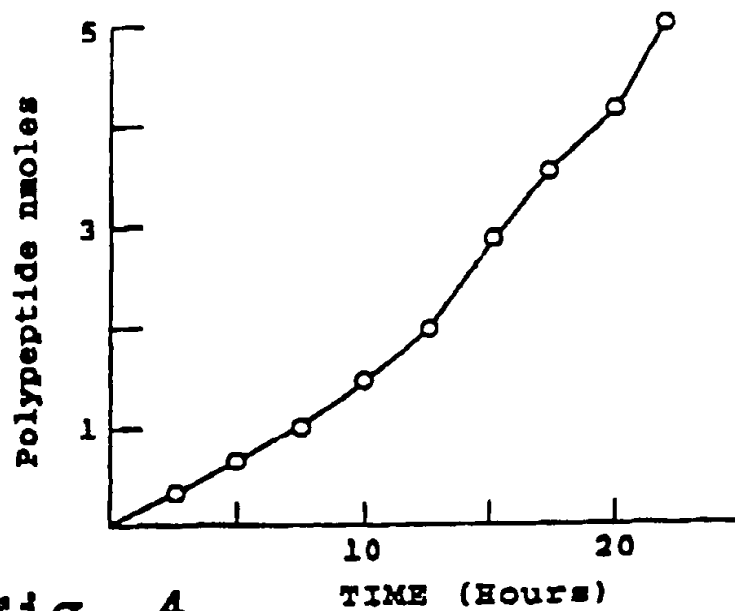


Fig. 4

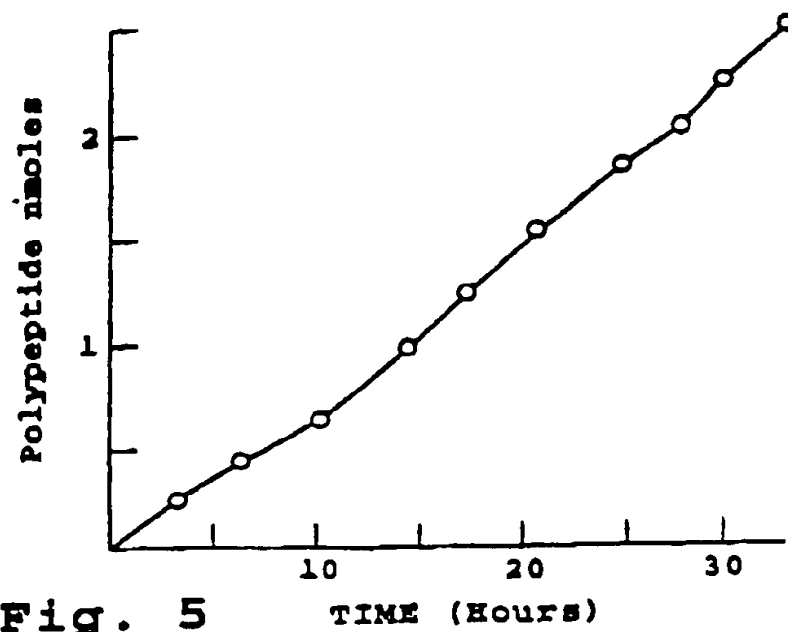


Fig. 5

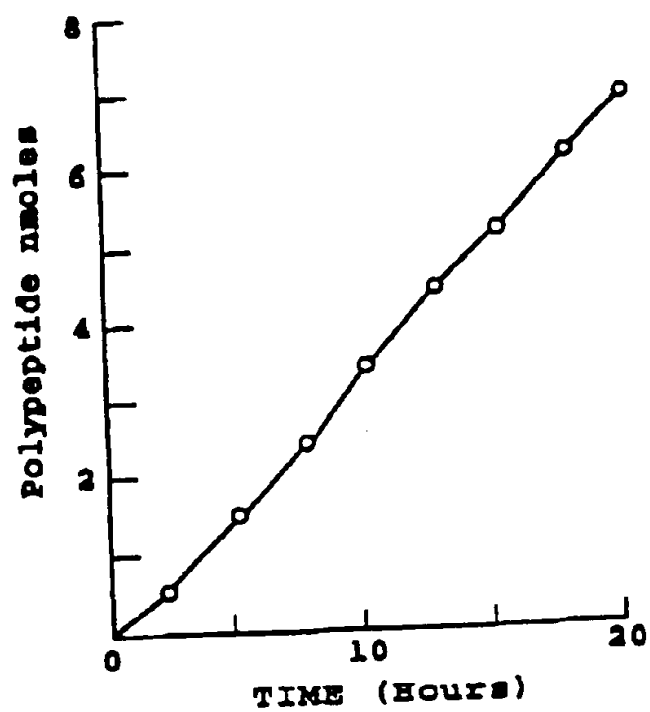


Fig. 7